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TRANSMITTAL FORM

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Total Number of Pages in This Submission

Application Number	09/991,796
Filing Date	11/23/2001
First Named Inventor	George Jackowski
Art Unit	1649
Examiner Name	Olga N. Chernyshev
Attorney Docket Number	2132.109

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
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- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):

Applicant's Brief in accordance with 37 CFR 41.37 w/Appendix
A copy of the Non-Compliance Notice dtd 12/14/06

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	McHale & Slavin, P.A.		
Signature	Ferris H. Lander		
Printed name	Ferris H. Lander		
Date	1/16/2007	Reg. No.	43,377

CERTIFICATE OF TRANSMISSION/MAILING

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

APPLICANT	: Jackowski et al.
INVENTION	: **Fibronectin Biopolymer Markers Indicative of Type II Diabetes**
SERIAL NUMBER	: 09/991,796
FILING DATE	: November 23, 2001
EXAMINER	: Chernyshev, Olga N.
GROUP ART UNIT	: 1649
OUR FILE NO.	: 2132.109

Mail Stop Appeal Brief-Patent
Commissioner for Patents
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Alexandria, VA 22313-1450

Dear Sir:

APPLICANT'S BRIEF IN ACCORDANCE WITH 37 C.F.R. § 41.37

Applicants submit this Appeal Brief to the Board of Patent Appeals and Interferences on appeal from the decision of Examiner Olga N. Chernyshev of Group Art Unit 1649 dated March 16, 2006, finally rejecting claim 1.

TABLE OF CONTENTS

I. REAL PARTY IN INTEREST

The real party in interest is Nanogen, Inc., the assignee of record.

II. RELATED APPEALS AND INTERFERENCES

A similar appeal has also been filed by Appellants in US Application Serial Number 09/994,909 (attorney docket number 2132.090), filed on November 23, 2001, which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending Appeal.

III. STATUS OF CLAIMS

Claims 1 and 39-46 are pending in the application. Claims 1-38 were originally presented. Claims 2-38 were cancelled without prejudice and new claims 39-46 were added by the amendment of September 22, 2003. Claims 39-46 were withdrawn from consideration on the merits based upon a restriction requirement. The final rejection of claim 1 under both 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph is appealed. Claim 1 is shown in the attached Claims Appendix.

IV. STATUS OF AMENDMENTS

No amendments have been filed subsequent to the Final Rejection mailed on March 16, 2006.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter relates to biopolymer markers, identified by the evaluation of a sample containing a plurality of biopolymers, which evidence a link to a specific disease state. See, specification at page 35, lines 14-18. Specifically, the biopolymer markers are SEQ ID NO:1 and SEQ ID NO:4 which evidence a link to Type II diabetes. Id. at page 46, line 14 to page 47, line 2 and Figures 1-4.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether Claim 1 is Unpatentable under 35 U.S.C. § 101 as Having No Specific and Substantial Credible Utility

1. Whether the Examiner Made a *Prima Facie* Showing that the Invention Lacks a Specific and Substantial Utility

2. Whether the Examiner Properly Held that Applicants' Asserted Utility Lacks Credibility

B. Whether Claim 1 is Unpatentable under 35 U.S.C. § 112, First Paragraph as Being Based on a Nonenabling Disclosure

1. Whether the Examiner Properly Evaluated the Application for Enablement.

VII. ARGUMENT

A. The Examiner Erred in Rejecting Claim 1 under 35 U.S.C. § 101

1. The Examiner Has Failed to Make a Prima Facie Showing that the Invention Lacks A Specific And Substantial Utility.

Claim 1, as shown in the attached Claims Appendix, stands finally rejected under 35 U.S.C. § 101. The Examiner maintains that the claimed invention is drawn to an invention with no apparent or disclosed specific and substantial credible utility.

Applicants respectfully traverse the rejection on the grounds that the application discloses an invention having specific, substantial, well-established and credible utility by showing an invention that is useful to the public as disclosed in its current form, rather than at some future date after further research, as peptide markers linked to Type II diabetes. Furthermore, Applicants have supported this utility with data specifically directed to patients having Type II diabetes.

The standard for satisfying the requirements for utility under 35 U.S.C. § 101 is not particularly high. In most cases, an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy 35 U.S.C. § 101. *See* In re Langer, 503 F.2d 1380, 1391, 183 USPQ 297 (CCPA 1974); MPEP § 2107.02(III)(A). In other words, the Office is correct to presume that a statement of utility made by an applicant is true.

Accordingly, the Examiner should presume that the claimed peptides (SEQ ID NOS:1 and 4) are useful as markers for Type II diabetes based upon Applicants' showing in Figures 1 and 3 that the peptides are linked to Type II diabetes by their differential expression in Type II diabetes patients as compared to healthy control patients.

A "specific utility" refers to a utility that is well-defined, particular and specific to the subject matter claimed. Vague expressions such as "a compound has useful biological activity" or "biological properties" are meaningless. In re Fisher, 421 F.3d 1365, 1371, 76 USPQ2d 1225 (Fed. Cir. 2005); In re Kirk, 376 F.2d 936, 941, 153 USPQ 48 (CCPA 1967); MPEP § 2107.01. For example, a general statement indicating that a marker is useful for diagnostics, such as diagnosing a disease, would be insufficient, absent a disclosure of what disease and/or condition could be diagnosed. In contrast, a statement of diagnostic utility, such as diagnosing Type II diabetes, would be sufficient to identify a specific utility for the invention. Thus, Applicants' statement of utility regarding the use of the claimed peptide as a marker for Type II diabetes constitutes a specific utility since the claimed peptide is linked to the specific condition of Type II diabetes.

It is well known that pathological changes in an organism are reflected by changes observed in the serum protein pattern. For example, proteins that undergo a change in expression (from the normal) are often indicative of disease. A diagnosis may be predicted based upon the similarity of unknown sample pattern to known pattern of disease. Mass spectrometry is a tool used to establish serum protein patterns.

Generally proteins, as collected from a serum sample, are too large to be effectively resolved by mass spectrometry and thus, are often first subjected to separation by polyacrylamide gel electrophoresis. Upon electrophoresis, the proteins contained in the sample separate into bands in specific areas of the gel according to weight and charge. The separated protein bands which are observed and deemed to be different between two comparable states (for example, disease state vs. normal state) are excised from the gel and subjected to further fragmentation by enzymes. The resulting peptides are then

collected and purified by chromatography prior to identification by mass spectrometry. The peptides undergo step-wise degradation into sequence-defining fragments, i.e. the peptides are part of the original protein found in the serum sample. The mass spectral profiles generated are composed of parts of the original protein and can be used to identify this protein.

In order for a rejection under 35 U.S.C. § 101 to be appropriate, the Examiner must demonstrate that there is a complete absence of data supporting the statements which set forth the desired results of the claimed invention. In re Cortright, 165 F.3d 1353, 1355, 49 USPQ2d 1464 (Fed. Cir. 1999).

It is respectfully submitted that the "link to Type II diabetes" asserted by Applicants was elucidated under real-world conditions according to the methodology set forth in the following steps:

I. isolating peptides from body fluid samples obtained from two groups of patients; a) one group known to suffer from Type II diabetes; and b) a group of controls (healthy in regard to Type II diabetes);

II. carrying out the protocols disclosed in the specification (pages 37-47);

III. comparing the expression pattern of protein bands from the two groups of patients as evidenced in gels (such as that shown in Figures 1 and 3);

IV. subjecting the noted expression pattern to the criteria as disclosed in the specification at page 11, lines 9-20;

V. selecting and excising bands that are differentially expressed between the two groups, and, submitting the peptides present within the excised bands for further fragmentation and purification followed by sequence identification by mass spectrometry.

The Applicants, using the above-described methodology in a real-world environment, thereby elucidated and identified SEQ ID NOS:1 and 4 as fragments of fibronectin precursor protein found in healthy, control patients but absent in patients having Type II diabetes, thus establishing the instantly claimed link to Type II diabetes evidenced by the observed differential expression.

The characteristic mass spectral profiles indicative of the claimed peptides are disclosed in Figures 2 and 4 (SEQ ID NO:1 in Figure 2 and SEQ ID NO:4 in Figure 4). Mass spectral profiles are reproducible and are typically published to provide established expression patterns for reference purposes.

Thus, any skilled artisan, in a real-world context, and without significant further research, could utilize the mass spectral profiles (shown in Figures 2 and 4) provided in the instant specification as references for comparing with mass spectral profiles of peptides obtained from an unknown sample to test the unknown sample for a link to Type II diabetes through comparison of expression patterns, thereby demonstrating that the specification discloses a specific and substantial utility for the claimed peptides. These mass spectral profiles are a showing of factual evidence that the claimed peptides could be used as markers for Type II diabetes. Thus, the instant specification provides data (gels shown in Figures 1 and 3 and the mass spectral profiles shown in Figures 2 and 4) supporting the desired results of the claimed invention; i.e. biopolymer markers for Type II diabetes.

Accordingly, Applicants respectfully submit that the Examiner has failed to adhere to the precedent set in Cortright by failing to establish that there is a complete

absence of data supporting the statements which sets forth the desired results of the claimed invention.

The Examiner asserts that one skilled in the art readily understands that in order to use the claimed peptides as markers for Type II diabetes, a point of reference that is critical for diagnosis with respect to the levels of differential expression of the claimed peptides must be disclosed. The Examiner then concludes that in the absence of this critical information, it is unclear as to how one of skill in the art can reasonably determine if the claimed peptides can be used as diagnostic markers for Type II diabetes and thus, one of skill in the art would have to resort to a substantial amount of further experimentation in order to practice Applicants' invention. However, with regard to providing a link to Type II diabetes as is instantly claimed, it is well settled that an applicant is not required to provide evidence of an asserted utility as a matter of statistical certainty. Nelson v. Bowler, 626 F.2d 853, 856, 206 USPQ 881 (CCPA 1980); MPEP § 2107.02.

Thus, Applicants respectfully submit that providing a point of reference that is critical for diagnosis with respect to the levels of differential expression of the claimed peptides is not necessary to establish credibility of the asserted use for the claimed peptides as markers for Type II diabetes. Accordingly, Applicants respectfully submit that the Examiner's requirement for such information is improper.

A "substantial utility" is a utility that defines a "real-world" use. MPEP §2107.01(I). "Substantial utility" refers to a significant and presently-available benefit to the public. An application must show an invention that is useful to the public as disclosed in its current form, not that it may prove useful at some future date after further research.

"In other words, one skilled in the art can use a claimed discovery in a manner which provides some immediate benefit to the public." Fisher, 421 F.3d at 1368, *citing* Nelson, 626 F.2d at 856.

In the context of an evaluation of substantial utility, the phrase "immediate benefit to the public" must not be interpreted to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. Brenner v. Manson, 383 U.S. 519, 534-535, 148 USPQ 689 (1966). Rather, any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient, at least with regard to defining a "substantial utility". MPEP § 2107.01(I).

Additionally, care must be given not to find a lack of specific and substantial utility based upon the setting in which the invention is to be used. This is particularly important in biotechnology; for example, during examination of inventions to be used in a research or a laboratory setting. As the Federal Circuit noted:

> "An assessment that focuses on whether an invention is useful only in a research setting thus does not address whether the invention is in fact 'useful' in a patent sense. [The PTO] must distinguish between inventions that have a specifically identified substantial utility and inventions whose asserted utility requires further research to identify or reasonably confirm." Fisher, 421 F.3d at 1372, *citing* MPEP § 2107.01(I).

Many research tools such as gas chromatographs, screening assays and nucleotide sequencing techniques have a clear, specific and unquestionable utility (e.g. they are useful in analyzing compounds). MPEP § 2107.01(I).

Furthermore, it is recognized that usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention becomes useful is well before

it is ready to be administered to humans. If Phase II testing was required in order to prove utility, the associated costs would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many crucial areas such as the treatment of cancer. *See* In re Brana, 51 F.3d 1560, 1568, 34 USPQ2d 1436 (Fed. Cir. 1995); MPEP § 2107.01(III).

The identification of the claimed peptides showing differential expression in Type II diabetes relative to healthy control patients puts a researcher one step closer to understanding the pathogenesis of Type II diabetes and thus, also one step closer to improved diagnosis and treatment of Type II diabetes. The mass spectral profiles of the claimed peptides can be used immediately to screen patient populations for links to Type II diabetes or the peptides can be used in further research to improve diagnosis and treatment of Type II diabetes. There is no question that improved diagnosis and treatment of Type II diabetes provides a tangible benefit to society; especially for the population susceptible to the development of Type II diabetes. Since the claimed peptides (SEQ ID NOS:1 and 4) have a "real-world" use in their currently available form as markers for Type II diabetes, i.e. the mass spectral profiles can be used to screen patient populations, the claimed peptides thus have a substantial utility.

Accordingly, there is a critical distinction between an invention that can be used in further experimentation and research, and an invention that requires further experimentation and research before it can be used. Applicants respectfully submit that the Examiner has erroneously found the claimed invention to be the latter rather than the former.

The Examiner cites Fisher in rejecting claim 1 and attempts to draw a parallel to the instant application by asserting that, just as in Fisher - where the Board reasoned that the use of the claimed expressed sequence tags ("ESTs") for the identification of polymorphisms is not a specific and substantial utility because "[w]ithout knowing any further information in regard to the gene represented by an EST, as here, detection of the presence or absence of a polymorphism provides the barest information in regard to genetic heritage," Fisher, 421 F.3d at 1368 - the detection of the claimed peptides in a sample of a patient suspected of having Type II diabetes provides no meaningful information as to the "link" or diagnosis determination.

Applicants respectfully submit that the facts in Fisher are inapposite to those concerning the present application. Fisher's invention related to five purified nucleic acid sequences – ESTs - obtained from the leaf tissue of maize plants. As described in Fisher, an EST is a short nucleotide sequence that represents a fragment of a cDNA clone. It is typically generated by isolating a cDNA clone and sequencing a small number of nucleotides located at one end of the two cDNA strands. When an EST is introduced into a sample containing a mixture of DNA, the EST may hybridize with a portion of the DNA. Such binding shows that the gene corresponding to the EST was being expressed at the time of mRNA extraction.

Fisher disclosed in his application that the claimed ESTs may have been used in a variety of ways, including, for example, measuring the level of mRNA in a tissue sample via microarray technology to provide information about gene expression, isolating promoters and identifying the presence or absence of a polymorphism. Fisher, 421 F.3d at 1368. However, Fisher made no disclosure regarding the precise structure or function of

either the genes or the proteins encoded for by those genes to which the claimed ESTs correspond. Id.

The Examiner of the Fisher application rejected the claims for lack of utility under 35 U.S.C. § 101 and lack of enablement under 35 U.S.C. § 112, first paragraph. The Board affirmed the rejections. In upholding the rejection, the Court cited the guidelines in MPEP § 2107.01(I) that state a claim to a polynucleotide whose use is disclosed simply as a "gene probe" or "chromosome marker" would not be considered to be specific in the absence of a disclosure of a specific DNA target. The Court noted the Applicants' admission that the underlying genes had no known functions, and that "[e]ssentially, the claimed ESTs act as no more than research intermediaries that may help scientists to isolate the particular underlying protein-encoding genes and conduct further experimentation on those genes". Id., at 1373. Accordingly, the Court found the ESTs to be mere "objects of use-testing", upon which scientific research could be performed with no assurance that anything useful will be discovered in the end. Id., *citing* Brenner, 383 U.S. at 535. Fisher's asserted uses represented merely hypothetical possibilities, objectives which the claimed ESTs, or any other EST for that matter, could possibly achieve, but none for which they have been used in the real world. For example, Fisher asserted that the ESTs could be used to identify polymorphisms or to isolate promoters. Nevertheless, in the face of a utility rejection, Fisher did not present any evidence showing that the ESTs had been used in either way. Id. Since nothing was known about the genes or proteins corresponding to the claimed ESTs, nothing set the claimed ESTs apart from the more than 32,000 ESTs disclosed in the application or from any EST derived from any organism. Id., at 1374. In other words, any EST could be

used to isolate any promoter. Furthermore, the use of the ESTs to actually identify the associated gene would constitute significant further experimentation, rendering the ESTs unable to be used in their current form. Ultimately, Fisher's ESTs were deemed only to be research intermediaries in the identification of underlying protein-encoding genes of unknown function. Id., at 1373.

In contrast to the invention of Fisher, the peptides (SEQ ID NOS: 1 and 4) of the instant invention are known to be fragments of fibronectin precursor protein having the amino acid sequences (i.e. structure) VDVIPVNLPGEHGQR (SEQ ID NO:1) and RVDVIPVNLPGEHGQRL (SEQ ID NO:4) . Furthermore, the claimed peptides are disclosed as markers of a specific disease condition, Type II diabetes. Any skilled artisan, without significant further research, could utilize the mass spectral profiles of the claimed peptides, shown in Figures 2 and 4, as references for comparison with mass spectral profiles obtained from an unknown sample to screen the sample for a link to Type II diabetes through comparison of expression patterns.

Thus, Applicants respectfully submit that the Examiner's attempt to draw a parallel between Fisher and the instant application fails to support her finding a lack of specific and substantial utility, as the facts in Fisher are not akin to the instant application.

It is clear, from consideration of all of the foregoing remarks, that the claimed invention has a specific and substantial utility. Thus, Applicants respectfully submit that the Examiner has failed to make a *prima facie* showing for lack of specific and substantial utility.

2. *The Examiner Improperly Finds Applicants' Asserted Utility Lacking in Credibility*

The Examiner does not doubt or dispute the results of differential expression of the instant claimed peptides of SEQ ID NOS:1 and 4. The main point of disagreement appears to be the interpretation of these results and what constitutes a specific, substantial and credible utility. Thus, the Examiner appears to believe that the showing of differential expression of the claimed peptides in Type II diabetes as compared to expression in healthy controls is not sufficient to indicate that the claimed peptides could be used as markers for Type II diabetes.

Applicants note that it is improper for Office personnel to merely question operability. Factual reasons must be set forth which would lead one of skill in the art to question the objective truth of the statement of operability. MPEP § 2107.02(IV).

The Examiner provides her opinion on what one of skill in the art would know. For example, the Examiner states that one skilled in the art readily appreciates that detection of differentially expressed proteins represents only the first step in identification of molecules that have a diagnostic potential and that one skilled in the art readily appreciates that many proteins are differentially expressed between healthy and "diseased" tissues (cancer cells, for example, overexpress a plurality of proteins by virtue of uncontrolled proliferation); however, not all of these proteins constitute biomarkers, as molecules that allow to distinguish disease vs. healthy state. However, the Examiner does not provide reasoning or references evidencing why one of skill in the art would "readily appreciate" these things.

Furthermore, the Examiner requires Applicant to provide complete characterization of the claimed peptides, including data indicating what level of differential expression of the claimed peptides is diagnostic of Type II diabetes, to establish a utility for the claimed peptides.

The instant situation is akin to that in Cortright. Cortright's invention was drawn to a method for treating baldness by applying Bag Balm (a commercially available product used to soften cow udders) to human scalp. The Examiner of the Cortright application rejected the claim drawn to this invention under 35 U.S.C. § 101 as lacking utility. According to the Examiner, Cortright's statement of utility, i.e. her claims of treating baldness, were not credible because baldness was generally accepted in the art as being incurable. The Examiner therefore required clinical evidence to establish the claimed utility, which Cortright did not supply. Cortright, 165 F.3d at 1355.

The Board reversed the rejection under 35 U.S.C. § 101 because the Examiner did not set out sufficient reasons for finding Cortright's statements of utility incredible. The Board additionally noted that there is no per se requirement for clinical evidence to establish the utility of any invention. Id.

Applicants respectfully submit that the Examiner has similarly erred by improperly questioning the operability of the invention, in that she states what one of skill in the art would believe without providing evidence to support her conclusion. Additionally, Applicants respectfully submit that the Examiner has further erred by requiring Applicants to provide "complete characterization" of the claimed peptide in order to establish utility since precedent dictates that evidence of absolute certainty is not required.

Compliance with 35 U.S.C. § 101 is a question of fact. Raytheon v. Roper, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983), *cert. denied*, 469 US 835 (1984); MPEP §2107.02(III)(A). Thus, to overcome the presumption of truth that an assertion of utility by the applicant enjoys, Office personnel must establish that it is more likely than not that one of ordinary skill in the art would doubt (i.e. "question") the truth of the statement of utility. MPEP § 2107.02(III)(A). Alternatively, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. MPEP § 2164.07(I)(C).

Furthermore, an Examiner must present countervailing facts and reasoning sufficient to establish that a person of ordinary skill in the art would not believe the applicants' assertion of utility. Brana, 51 F.3d at 1568; MPEP § 2107.01(III).

In the prior art, the showing of a link between a peptide and a disease implies the potential for use of the peptide for diagnosis and/or therapeutics of the disease. For example, Blennow et al. (Dementia 6(6):306-311 1995, copy attached hereto in the Evidence Appendix) suggest, based upon differential expression, that chromogranin in cerebrospinal fluid has a potential as a biochemical marker in Alzheimer's disease (Type I, pure AD). Since these practices are common, it is reasonable to believe that when one of skill in the art observes the differential expression of the claimed peptides between Type II diabetes patients and healthy control patients; one of skill in the art would, more likely than not, connect these peptides with potential diagnostics and/or therapeutics for Type II diabetes.

Furthermore, Applicants respectfully submit that one of ordinary skill in the art would find the suggestion of a link between the claimed peptides (SEQ ID NOS:1 and 4),

fibronectin, and Type II diabetes to be reasonable because there is a known association between fibronectin and Type II diabetes.

Fibronectin is a key component of the extracellular matrix; functioning, through a series of binding domains, to maintain normal cell morphology via organization of cell attachment to the extracellular matrix. Fibronectin is particularly prone to fragmentation since the regions between the binding domains are highly susceptible to proteolysis. Fibronection fragments are known to have functions not found in the intact protein, such as exerting affects on the proliferation and migration of endothelial cells. *See* Grant et al. Diabetes 47:1335-1340 1998, copy attached hereto in the Evidence Appendix.

Additionally, increased proteolysis is known to contribute to the pathologic process of Type II diabetes. *See* comment by Luc Tappy on Gastadelli et al. Diabetes 49:1367-1373 2000, copy attached hereto in the Evidence Appendix.

Furthermore, excess fibronectin produced in diabetes is theorized to be available for fragmentation. Grant et al. hypothesized that the formation of abnormal fibronectin fragments *in vivo* could facilitate aberrant angiogenesis, as seen in such conditions as proliferative diabetic retinopathy. *See* Grant et al. Id.

The claimed peptides (SEQ ID NOS:1 and 4), elucidated from and differentially expressed in diseased versus normal samples, are identified as fragments of fibronectin precursor protein at page 46, line 14 to page 47, line 2 of the specification as originally filed and are consistently replicated in the sample population. The gels shown in Figures 1 and 3 demonstrate that these peptides are found to be expressed in normal patients but absent in Type II diabetes patients. This data is consistent with the studies indicating the involvement of fibronectin in the pathogenesis of diabetes. Thus, the instant inventors

hypothesized that expression of the fibronectin precursor fragments in patients considered to be normal with regard to Type II diabetes when compared to expression seen in patients with a history of Type II diabetes indicates that fragmentation of fibronection may occur during the diabetic disease process. One of skill in the art, considering the known association of fibronectin and diabetes, would find such a hypothesis to be reasonable.

Considering that there is a known increase in proteolysis in Type II diabetes and that fibronectin is particularly sensitive to such proteolysis (degradation into fragments) and further considering the suggestion in the prior art that fibronectin fragments may be involved in pathogenic diabetic processes such as proliferative retinopathy, a skilled artisan would find Applicants' hypothesis and the data disclosed in the specification entirely plausible, and thus would reasonably link the claimed peptides (SEQ ID NOS:1 and 4) with Type II diabetes.

One of ordinary skill in the art would conclude, based upon all of the foregoing remarks, that the asserted utility for the claimed peptides, use as markers for Type II diabetes, is more likely than not true. Thus, Applicants respectfully submit that the Examiner has failed to make a *prima facie* case for lack of credible utility.

B. The Examiner Erred in Rejecting Claim 1 under 35 U.S.C. § 112, First Paragraph.

1. The Examiner Improperly Finds the Invention Nonenabled

Claim 1, as shown in the attached Claims Appendix, stands finally rejected under 35 USC § 112, first paragraph.

It is well established that the enablement requirement of 35 U.S.C. § 112 incorporates the utility requirement of 35 U.S.C. § 101. Fisher, 421 F.3d at 1378. Where a written description fails to illuminate a credible utility, the PTO will make both a Section 112 rejection for failure to teach how to use the invention and a Section 101 rejection for lack of utility. Cortright, 165 F.3d at 1355. "If [certain] compositions are in fact useless, [a] specification cannot have taught how to use them." Id..

In most cases, an applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101. As the Court of Customs and Patent Appeals stated in In re Langer:

> As a matter of Patent Office practice, a specification which contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented must be taken as sufficient to satisfy the utility requirement of § 101 for the entire claimed subject matter unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope. Langer, 503 F.2d at 1391 (emphasis in original).

The "Langer" test for utility has been used in evaluation of rejections under 35 U.S.C. § 112, first paragraph, where the rejection is based on a deficiency under 35 U.S.C. § 101. An examiner cannot make this type of rejection, however, unless it has reason to doubt the objective truth of the statements contained in the written description. Cortright, 165 F.3d at 1357. A reason to doubt an asserted utility may be established when the description "suggests an inherently unbelievable undertaking or involves implausible scientific principles." Brana, 51 F.3d at 1566.

In the present application, the Examiner rejects Claim 1 under 35 U.S.C. § 112, first paragraph, "since the claimed invention is not supported by either a clear asserted utility or well established utility for the reasons set forth [in the Examiner's rejection

under 35 U.S.C. § 101] . . . one skilled in the art clearly would not know how to use the claimed invention." Applicants respectfully traverse the rejection as Applicants have established in the above remarks that the claimed invention has a specific and substantial credible utility.

A skilled artisan could easily follow the methodology for elucidating the presence of the claimed peptides (SEQ ID NOS: 1 and 4), as disclosed in the patent application (and reiterated *supra*), on a non-differentiated patient population, in order to discern members of the population who manifest Type II diabetes.

Thus, one of skill in the art clearly would know how to use the claimed peptides (SEQ ID NOS: 1 and 4) as markers for Type II diabetes. Thus, Applicants respectfully submit that the Examiner has failed to properly establish lack of enablement.

VIII. CONCLUSION

In conclusion, in light of the foregoing, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case for lack of utility and lack of enablement in the present application. Favorable reconsideration of this application and withdrawal of the rejections of claim 1 under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph is courteously requested.

Respectfully submitted,

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IX. CLAIMS APPENDIX

Claim 1. An isolated biopolymer marker which evidences a link to Type II diabetes selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:4.

X. EVIDENCE APPENDIX

A. Appellants rely on two declarations under 37 C.F.R. § 1.132.

1. The Declaration under 37 C.F.R. §1.132, filed on September 22, 2003, was entered into the prosecution record by the Examiner at page 6 of the Office Action mailed on March 9, 2004.

2. The Declaration under 37 C.F.R. § 1.132, filed on February 9, 2006, was entered into the prosecution record by the Examiner at page 8 of the Office Action mailed on March 16, 2006.

B. Appellants rely on three references, all previously presented to the Examiner in the Response filed on February 9, 2006.

1. Blennow et al. Dementia 6(6):306-311 1995

2. Grant et al. Diabetes 47:1335-1340 1998

3. Luc Tappy on Gastadelli et al. Diabetes 49:1367-1373 2000

Copies of the above-referenced declarations and references are attached hereto as forms the Evidence Appendix.

XI. RELATED PROCEEDINGS APPENDIX

NONE.

There have been no decisions rendered by a court or the Board in the related proceeding identified at Section II, page 5, of this paper.

COPY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICANT	: Jackowski *et al.*
INVENTION	:**Fibronectin Biopolymer Markers Indicative Of Type II Diabetes**
SERIAL NUMBER	: 09/991,796
FILING DATE	: November 23, 2001
EXAMINER	: Chernyshev, Olga N.
GROUP ART UNIT	: 1646
OUR FILE NO.	: 2132.109

CERTIFICATE UNDER 37 CFR 1.8(a)
I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 on 9-16-2003

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Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

I, Dr. George Jackowski, do hereby declare as follows:

1. I am Chief Executive Officer and Chief Science Officer of Syn-x Pharma Inc., assignee in the application entitled **"Fibronectin Biopolymer Markers Indicative Of Type II Diabetes"**, having U.S. Application Serial No. 09/991,796, filed November 23, 2001.

2. In the Office Action mailed on June 16, 2003, claims 1 and 2 (as originally presented) were rejected under 35 U.S.C. 112, first paragraph because the claimed invention allegedly contains subject matter which was not described in the specification in such

a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner states that the invention is directed to a biopolymer marker of SEQ ID NO:1 or SEQ ID NO:4 or at least one analyte thereof useful in indicating at least one particular disease, for example, Type II Diabetes. The Examiner asserts that the specification fails to provide any guidance on how to use the disclosed peptides (SEQ ID NO:1, SEQ ID NO:4 and analytes thereof) as markers or indicators of any disease, including Type II Diabetes. The Examiner is particularly concerned with an alleged lack of controls in the experiments.

3. This declaration is submitted in order to clarify the use of controls in the experiments disclosed in the specification.

4. There are no conventional controls applied in the methods of the instant invention. Both samples from diseased patients and samples from healthy patients are separated by polyacrylamide gel electrophoresis. The gel is then examined in order to identify differences in the bands appearing in diseased and healthy patients. The bands, which differ between healthy and diseased patients, are excised and purified from the gel. A determination of upregulation, downregulation, presence and/or absence of the proteins present in the bands is assessed by sample wherein they appear, for example, the claimed peptide fragments were identified and excised from bands which appeared lighter in the diseased sample as compared with the healthy sample, indicating a decreased amount of protein expression or protein degradation in the diseased

samples. Thus, this is considered to be downregulation of the protein in the disease sample as compared to the higher level of expression of the protein in the healthy sample. This comparison between two physiological states as evidenced by the bands appearing on the gel represents an inherent control in the experiment. The claimed protein fragments excised from the band appearing in the diseased sample are sequenced and identified through the application of mass spectrometric techniques. Since the band appeared darker in the healthy sample, the corresponding lighter band in the diseased was chosen for excision and sequencing. It is standard laboratory practice to sequence peptides by mass spectrometry and identify the peptides based upon known sequences available in databases; thus sequencing and comparison of control peptides is not required. One of ordinary skill in the art would be familiar with these standard protocols of mass spectrometry.

The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application or any patent issuing thereon.

Sept 15 2003.
Date

George Jankowski

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICANT	: Jackowski *et al.*
INVENTION	: **Fibronectin Biopolymer Markers Indicative of Type II Diabetes**
SERIAL NUMBER	: 09/991,796
FILING DATE	: November 23, 2001
EXAMINER	: Chernyshev, Olga N
GROUP ART UNIT	: 1649
OUR FILE NO.	: 2132.109

CERTIFICATE UNDER 37 CFR 1.8(a)
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Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

I, Ferris H. Lander, do hereby declare as follows:

1. I am a registered Patent Agent and am authorized to represent the inventor's and assignee in the application entitled **"Fibronectin Biopolymer Markers Indicative of Type II Diabetes"**, having U.S. Application Serial No. 09/991,796, filed November 23, 2001.

2. In the Office Action mailed on November 4, 2005, claim 1, (as presented on May 16, 2005) was rejected under 35 USC 101 because the claimed invention allegedly has no apparent or

disclosed specific and substantial credible utility. Claim 1 was also rejected under 35 USC 112, first paragraph since the claimed invention is not supported by either a clear asserted utility or a well established utility, according to Examiner, one skilled in the art clearly would not know how to use the claimed invention.

Specifically, the Examiner asserts that the specification does not support Applicants' statement that the presence of the claimed biopolymer markers (SEQ ID NOS:1 and 4) in a sample is indicative of a link to Type II diabetes. The Examiner maintains that the claimed biopolymer markers (SEQ ID NOS:1 and 4) are only useful for further research.

3. Applicants submit that Figures 1-4, as originally filed, are "evidence of record" which supports Applicants' possession of the claimed peptides (SEQ ID NOS:1 and 4) and their relationship to Type II diabetes.

(a) Figure 2 (as originally filed) shows a mass spectral profile obtained from Band 1 of the gel shown in Figure 1. Expression of the claimed peptide (SEQ ID NO:1) was shown, in Figure 1, to be present in serum samples obtained from normal patients and not present in serum samples obtained from Type II diabetes patients. Thus, the claimed peptide (SEQ ID NO:1) is differentially expressed in Type II diabetes versus normal.

(b) Figure 4 (as originally filed) shows a mass spectral profile obtained from Band 1 of the gel shown in Figure 3. Expression of the claimed peptide (SEQ ID NO:4) was shown, in Figure 3, to be present in serum samples obtained from normal

patients and not present in serum samples obtained from Type II diabetes patients. Thus, the claimed peptide (SEQ ID NO:4) is differentially expressed in Type II diabetes versus normal.

4. (a) In order to further illustrate this point, Applicants provide the attached figure entitled "DEAE 1(Elution) Normal vs. Diabetes Type II" which represents Figure 1 as originally filed. The attached figure was produced by scanning the original photograph of the gel. Expression of Band #1 is shown in samples obtained from patients determined to be normal with regard to Type II diabetes (lanes 1-4, as read from the left) and is not shown in samples obtained from Type II diabetes (lanes 5-9). Thus, the claimed peptide (SEQ ID NO:1) is shown to be differentially expressed between Type II diabetes and normal controls. No new matter has been added; this figure is simply a clearer copy of Figure 1 as originally filed and is provided to clarify the presence and differential expression of the claimed biopolymer marker (SEQ ID NO:1). The gel shown in the figure does not represent new experimentation; the figure shows a clearer image of the original gel made at the time that the experiments described in the instant specification were first carried out.

(b) In order to further illustrate this point, Applicants also provide the attached figure entitled "HiQ3 (scrub) Normal vs. Diabetes Type II" which represents Figure 3 as originally filed. The attached figure was produced by scanning the original photograph of the gel. Expression of Band #1 is shown in samples obtained from patients determined to be normal with regard to Type

II diabetes (lanes 7-10, as read from the left) and is not shown in samples obtained from Type II diabetes (lanes 2-6). Thus, the claimed peptide (SEQ ID NO:4) is shown to be differentially expressed between Type II diabetes and normal controls. No new matter has been added; this figure is simply a clearer copy of Figure 3 as originally filed and is provided to clarify the presence and differential expression of the claimed biopolymer marker (SEQ ID NO:4). The gel shown in the figure does not represent new experimentation; the figure shows a clearer image of the original gel made at the time that the experiments described in the instant specification were first carried out.

The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application or any patent issuing thereon.

2/6/2006.
Date

Ferris H. Lander
Reg. No. 43,377

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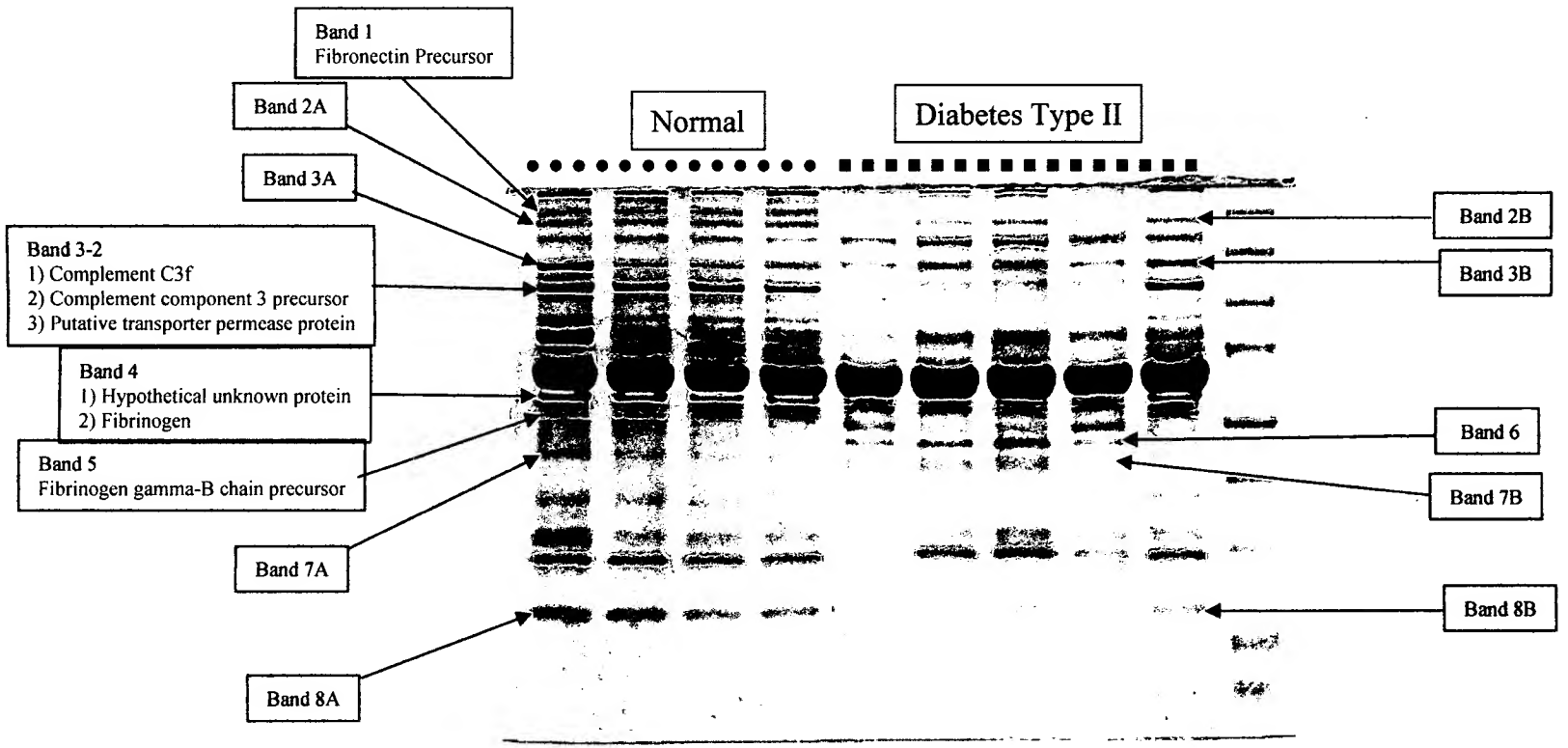
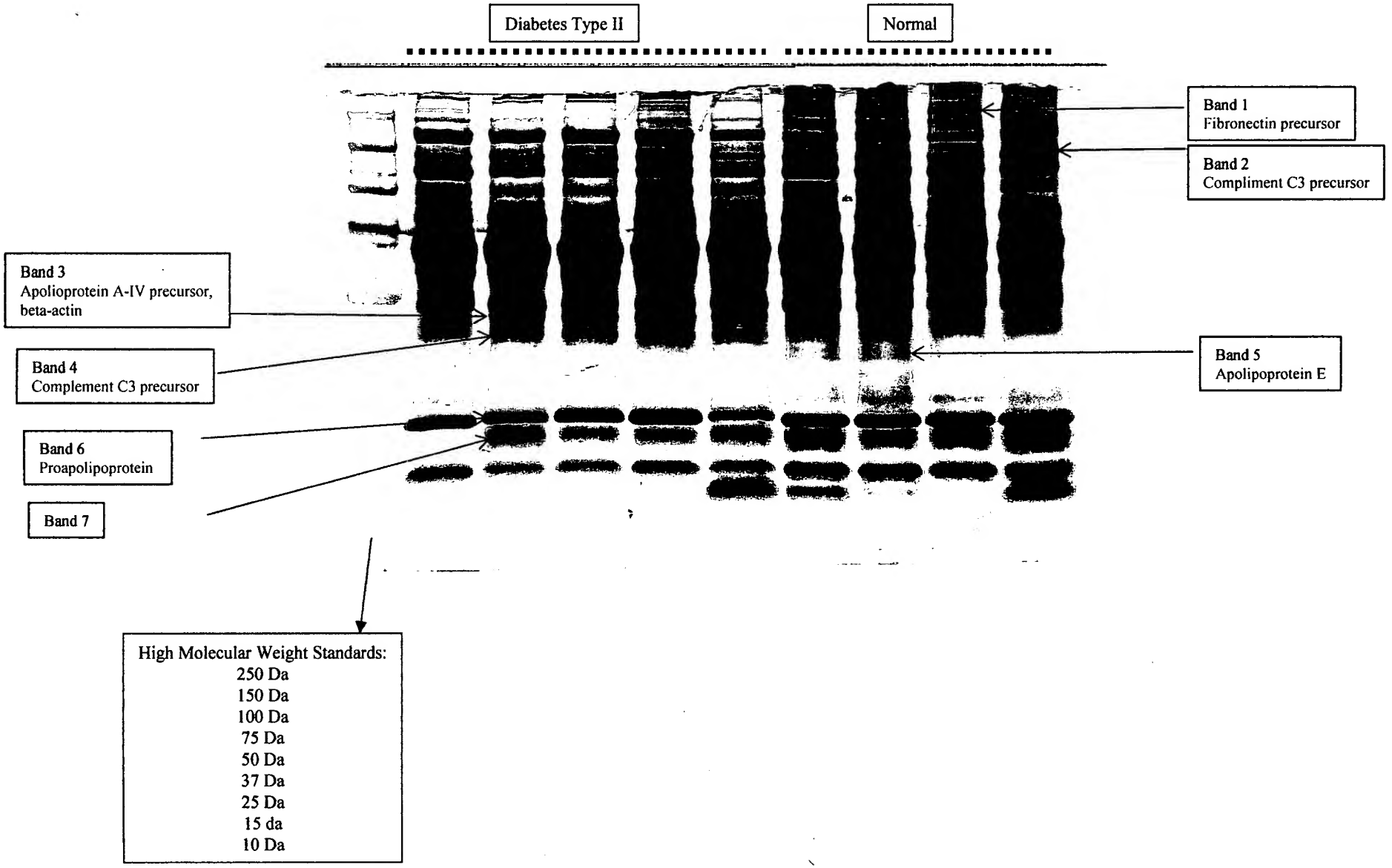
DEAE 1(Elution) Normal vs. Diabetes Type II



HiQ3 (scrub) Normal vs. Diabetes Type II



1: Dementia. 1995 Nov-Dec;6(6):306-11.

Links

Chromogranin A in cerebrospinal fluid: a biochemical marker for synaptic degeneration in Alzheimer's disease?

Blennow K, Davidsson P, Wallin A, Ekman R.

Department of Psychiatry and Neurochemistry, University of Goteborg, Molndal Hospital, Sweden.

Biochemical markers for AD would be of great value both to improve the clinical diagnostic accuracy in scientific studies and to increase the knowledge of the pathogenesis of the disorder. One of the main features of AD is a degeneration of synapses. Therefore, we examined if chromogranin A (CrA), the major protein of large dense-core synaptic vesicles, in cerebrospinal fluid (CSF) may be of value as a biochemical marker for the synaptic function in AD. The mean concentration of CrA in CSF was about 7.5 times higher than its concentration in serum, and there was no significant correlation between CSF-CrA and the blood-brain barrier function (measured as the CSF/serum albumin ratio), nor between CSF-CrA and serum-CrA. These findings suggest that the major portion of CSF-CrA is locally produced within the CNS. There were no significant differences in CSF-CrA between the AD (n = 29), vascular dementia (n = 13), and age-matched control (n = 9) groups (99.9 +/- 58.9 ng/ml, 108.0 +/- 69.4 ng/ml, and 115.1 +/- 44.4 ng/ml, respectively). However, when the AD group was subdivided into AD type I (n = 12) and AD type II (n = 17), a lower concentration of CSF-CrA was found in AD type I (72.8 +/- 28.9 ng/ml) compared with controls (115.1 +/- 44.4 ng/ml), $p < 0.02$, and compared with AD type II (119.1 +/- 67.5 ng/ml), $p < 0.05$, while CSF-CrA did not significantly differ between AD type II and controls. These findings suggest that CSF-CrA has a potential as a biochemical marker for the synaptic degeneration in AD type I, and gives further support for the relevance of identifying the AD type I (pure AD) subgroup in scientific studies.

PMID: 8563783 [PubMed - indexed for MEDLINE]

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Fibronectin Fragments Modulate Human Retinal Capillary Cell Proliferation and Migration

Maria B. Grant, Sergio Caballero, David M. Bush, and Polyxenie E. Spoerri

Capillary morphogenesis involves cell-cell and cell-matrix interactions. Proteases elaborated by capillary cells modify the extracellular matrix (ECM) to facilitate capillary tube formation. Previously, we detected the presence of fibronectin fragments (Fn-f) associated with the proform of matrix metalloprotease-2 (MMP-2) in conditioned medium of human retinal endothelial cells (HRECs). Association of this fragment to latent MMP-2 prevented autocatalytic activation of MMP-2, suggesting a modulatory role of Fn-f in MMP-2 activation. In this report, we examined the potential role of Fn-f on two processes involved in angiogenesis, proliferation and migration of vascular cells. The effects of Fn-f on proliferation were determined by DNA synthesis and cell counts. Their effects on migration were assessed using modified Boyden chambers. Seven Fn-f were tested on vascular cell migration and/or proliferation. Three Fn-f induced migration. Fn-f of 30-kDa and 120-kDa size positively affected proliferation of microvascular cells but not macrovascular cells. A 45-kDa gelatin binding fragment of Fn inhibited HREC proliferation but stimulated pericyte and smooth muscle cell proliferation. The potency of these fragments exceeded that of the known angiogenic growth factor, basic fibroblast growth factor (bFGF), on HREC migration. ECM components such as fibronectin may influence capillary morphogenesis by the generation of fragments that can modulate proliferation, migration, and protease activation. In the setting of diabetes, excess Fn is generated and is available for degradation. Thus, the production of Fn-f may be specifically relevant to the angiogenesis observed in proliferative diabetic retinopathy. *Diabetes* **47:1335–1340, 1998**

Intrinsic to angiogenesis is the migration, proliferation, and formation of capillary tubes by endothelial cells. Quiescent endothelial cells become activated by soluble mitogens and insoluble extracellular matrix (ECM) molecules producing proteases for the degradation of matrix proteins to facilitate capillary tube formation (1). Fibronectin (Fn), a high-molecular-weight, adhesive, multifunctional glycoprotein and a key ECM component, has diverse biological activities (2). Fn exerts growth factor and differentiated activities in many types of cells and plays a vital role in cellular adhesion and migration, oncogenic transformation, wound healing, and hemostasis (3).

One of the most important functions of Fn is the maintenance of normal cell morphology via organization of cell attachment to the ECM. This is accomplished by a series of binding domains, including fibrin, factor XIIa, gelatin/collagen, DNA, heparin, and cell binding domains. The regions between these domains are highly susceptible to proteolysis, which gives rise to fibronectin fragments (Fn-f). Fn-f have been found to have activities not found in the intact molecule, and selected Fn-f have been shown to affect proliferation (4) and stimulate migration (5,6).

In a previous study, we found that human retinal endothelial cells (HRECs) of diabetic origin and nondiabetic HRECs after exposure to glucose expressed a novel proteolytic activity that migrated at 90 kDa. This 90-kDa activity represented the matrix metalloprotease-2 (MMP-2) tightly associated with Fn-f, and association of this fragment inhibited the autoactivation of MMP-2 (7). In the present study, the effect of selected Fn-f on vascular cell proliferation and migration was examined. This study supports that the generation of Fn-f may regulate microvascular cell behavior and that abnormal Fn-f formation in vivo could facilitate aberrant angiogenesis, as seen in proliferative diabetic retinopathy.

RESEARCH DESIGN AND METHODS

Cell cultures. Human eyes were obtained from the National Disease Resource Interchange within 36 h of death. The eyes were dissected, and the retinas were removed and digested. HREC cultures were prepared and the purity of the culture assessed as previously described (8). HRECs were routinely seeded at 6 × 10^3 cells/cm^2 in 75-cm^2 flasks. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air.

In certain studies, cells were treated with whole Fn (cellular Fn; Sigma, St. Louis, MO) from human foreskin fibroblasts or with the following Fn-f: 30-kDa tryptic (Sigma), which spans a small segment of the terminal end of the heparin II/fibrin I domain and continues into the collagen/gelatin binding domain; 40-kDa chymotryptic (Life Technologies, Gaithersburg, MD), comprising a small segment of the COOH-terminal end of the heparin I/cell binding domain and continues into the heparin III binding domain; 45-kDa tryptic (Sigma), which comprises most of the collagen/gelatin binding domain; 70-kDa cathepsin D (Sigma), which contains most of the heparin II/fibrin I as well as the collagen/gelatin binding domains; 110-kDa chymotryptic (Upstate Biotechnology, Lake Placid, NY), containing the cell binding (but not heparin I) domain; and 120-kDa chymotryptic (Life Technologies), which spans a small portion of the collagen/gelatin binding domain and nearly all of the heparin I/cell binding domain. All fragments were purified by high-performance liquid chromatography, reconstituted according to the manufacturer's instructions, and stored at −80°C in single-use aliquots. All fragments were analyzed by SDS-PAGE and confirmed as single bands by silver staining. To further confirm purity of Fn-f, NH_2-terminal sequencing was performed on the 120-kDa, 45-kDa, and 30-kDa proteins by the Protein Sequencing Core at the University of Florida.

From the Division of Endocrinology and Metabolism, Department of Medicine, University of Florida, Gainesville, Florida.

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Received for publication 11 November 1997 and accepted in revised form 22 April 1998.

BFGF, basic fibroblast growth factor; BrdU, bromodeoxyuridine; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; Fn, fibronectin; Fn-f, fibronectin fragment(s); HREC, human retinal endothelial cell; HUVEC, human umbilical vein endothelial cell; MMP-2, matrix metalloprotease-2; SMC, smooth muscle cell; tPA, tissue-type plasminogen activator.

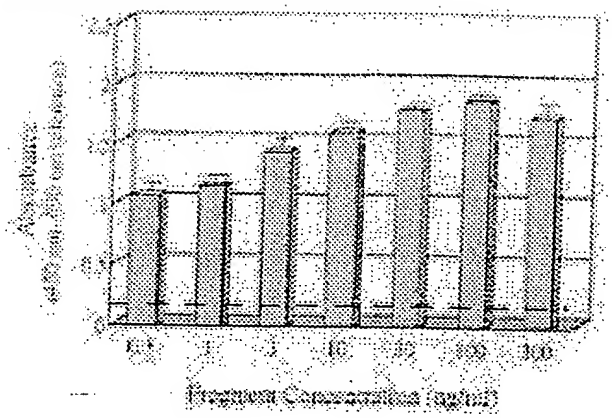

FIG. 1. HREC proliferation in response to increasing doses of the 120-kDa tryptic Fn-f. Cells were made quiescent by withdrawing serum for 18 h before the addition of 120-kDa Fn-f at the doses indicated. After an additional 24 h, the last 4 of which BrdU labeling reagent was added, the cells were processed according to manufacturer's directions to measure BrdU incorporation. Data are expressed as optical density at the wavelength indicated. The dashed line denotes the mean absorbance of untreated cells. Each bar is the mean of quadruplicate determinations, with standard error indicated by the error bar. BrdU incorporation reaches a plateau at 100 ng/ml ($P < 0.05$ for each dose up to 100 ng/ml vs. the previous dose; NS for 300 vs. 100 ng/ml).

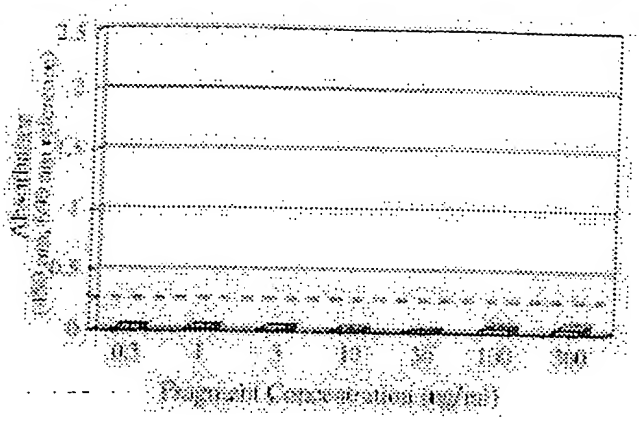

FIG. 2. Response of HUVECs to increasing doses of the 120-kDa chymotryptic Fn-f. These cells and the HRECs were treated identically. Data are expressed as optical density at the wavelength indicated. The dashed line denotes the mean absorbance of untreated cells. Each bar is the mean of quadruplicate determinations, with standard error indicated by the error bar. There is no dose-dependent trend in BrdU incorporation in these cells.

Human coronary artery smooth muscle cells (SMCs) were isolated and cultured as described (9). Human retinal pericytes were isolated at the time of HREC isolation and cultured separately as described (10). Human umbilical vein endothelial cells (HUVECs) were kindly provided by Dr. T. Maciag.

Proliferation. Bromodeoxyuridine (BrdU) incorporation (Boehringer Mannheim, Indianapolis, IN) was used to measure cell proliferation in response to various doses of Fn-f in HRECs, HUVECs, SMCs, and pericytes. Cells were grown in 96-well microtiter plates to ~50% confluence. They were then exposed to each Fn-f at the described doses for the indicated time of either 12 (HREC only), 24, 48, or 72 h. All treatments were performed in triplicate. Proliferation was indicated by a change in absorbance after reaction with anti-BrdU antibody and a colorimetric substrate reaction, as outlined by the manufacturer, and reported as fold change versus untreated cells. Based on the results of these studies, three Fn-f (30, 45, and 120 kDa) were selected for further examination as described below.

Migration studies. Chemotaxis studies were performed as previously described using modified Boyden chambers (11). For these studies, 25 µl of a suspension of HREC (1.6×10^6 cells/ml) was placed in each well of the inverted blindwell apparatus containing 48 wells. Wells were overlaid with a porous (5 µm diameter pores) polyvinyl-free and pyrrolidine-free polycarbonate membrane (Nuclepore, Pleasanton, CA), coated with 40 µg/ml bovine dermal collagen (Sigma). After allowing the cells to adhere to the membrane, chambers were then placed upright and test substances added (50 µl/well). Each Fn-f or whole Fn was tested at the indicated concentration. Dulbecco's modified Eagle's medium (DMEM) served as negative control, and DMEM containing 10% fetal calf serum served as positive control to assess chemotaxis. Chemokinesis, the nonoriented increase in cell locomotion in response to a stimulus, was measured for each Fn-f by adding equal concentrations of each fragment being tested to both upper and lower wells to abolish the concentration gradient. Chambers were disassembled after the specified time, cells on the attachment side were scraped off, and membranes were stained for analysis as previously described (11). Cells that migrated through the membrane were counted for each well and reported as cells per high power field. Additional chemotaxis experiments were performed comparing the effect of eqimolar concentrations of Fn-f and basic fibroblast growth factor (bFGF), since bFGF is a known stimulator of migration.

RESULTS

Proliferation. Of the five Fn-f examined, only three affected proliferation in HRECs. The 30-kDa and 120-kDa fragments induced proliferation as assessed by increased BrdU incorporation and cell number. Addition of the 120-kDa Fn-f resulted in a dose-dependent increase in BrdU incorporation in HRECs. Even at the lowest concentration examined, there was a 40-fold increase over basal, reaching maximal effect at 100 ng/ml (Fig. 1). When HUVECs were exposed to varying concentrations of the 120-kDa fragment, no change in BrdU incorporation was observed at any concentration tested (Fig. 2). The 30-kDa fragment resulted in a dose-dependent increase in BrdU incorporation. The maximal effect was achieved at 10 ng/ml. Higher concentrations resulted in stimulation, but not as great as at 10 ng/ml (Fig. 3). The 45-kDa fragment inhibited BrdU incorporation in HREC compared with untreated HREC (Fig. 3). However, the 45-kDa Fn-f increased BrdU incorporation in a dose-dependent manner in SMCs and pericytes (Fig. 4*A* and *B*). A maximal six- to sevenfold increase in BrdU incorporation was observed with both pericytes and SMCs, in contrast to the 20-fold decrease seen with this fragment in HRECs. The 30-kDa and the 120-kDa fragments increased BrdU incorporation

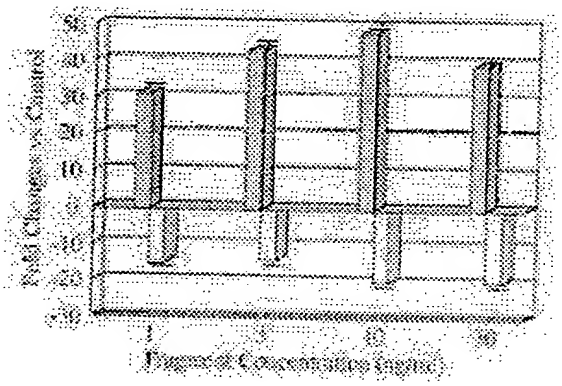

FIG. 3. HREC proliferation in response to increasing doses of the 30-kDa tryptic or 45-kDa tryptic Fn-f. Cells were treated identically to HRECs exposed to the 120-kDa Fn-f. Data were normalized to untreated cells and expressed as fold change. Each bar is the mean of quadruplicate determinations, with standard error indicated by the error bar. The 30-kDa fragment (▨) exhibits a dose-dependent increase in BrdU incorporation that reaches a plateau at 30 ng/ml ($P < 0.05$ for each dose up to 10 ng/ml vs. the previous dose; NS for 30 vs. 10 ng/ml). The 45-kDa fragment (□) exhibits a dose-dependent decrease in BrdU incorporation in these cells ($P < 0.05$ for each dose up to 30 ng/ml vs. the previous dose).

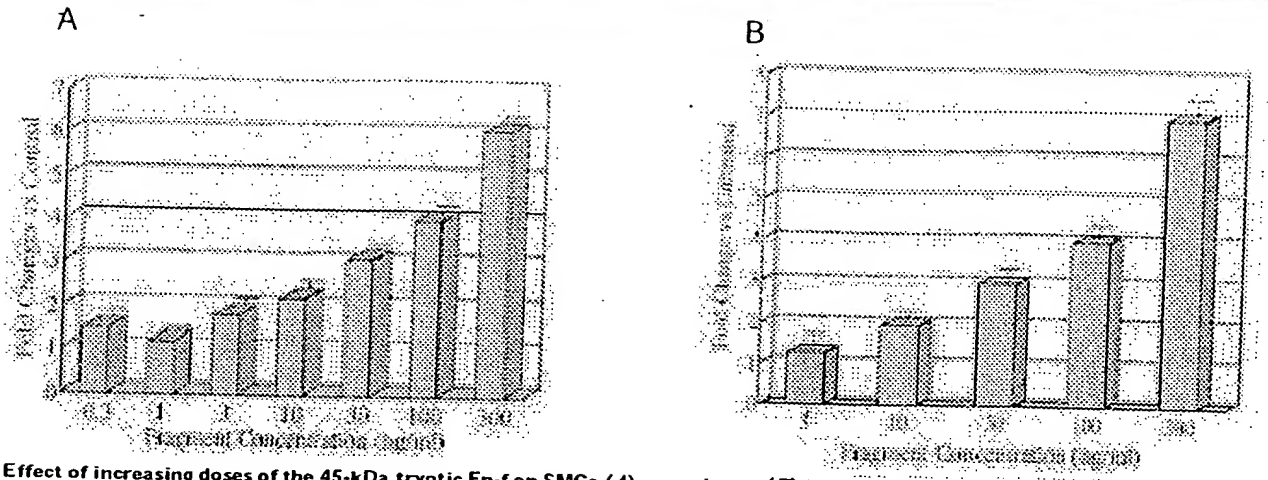

FIG. 4. Effect of increasing doses of the 45-kDa tryptic Fn-f on SMCs (*A*) or pericytes (*B*) in culture. Cells were treated identically to HRECs exposed to the 120-kDa Fn-f. Data were normalized to untreated cells and expressed as fold change. Each bar is the mean of quadruplicate determinations, with standard error indicated by the error bar. *A*: The 45-kDa Fn-f induces DNA synthesis in cultured SMCs in a dose-dependent fashion, showing a sixfold change versus untreated cells at the highest dose tested ($P < 0.05$ for each dose from 30 to 300 ng/ml vs. the previous dose; NS for 0.3 vs. 1 ng/ml). *B*: Cultured pericytes also show a dose-dependent increase in DNA synthesis in response to the 45-kDa Fn-f, with a sevenfold increase at the highest dose tested ($P < 0.05$ for each dose vs. the previous dose).

two- to threefold in SMC (data not shown) in contrast to the 35- to 70-fold increases observed in HREC.

The effect of exposure to Fn-f on cell number in HREC was examined. Cell number at 24 h rose in a dose-dependent manner by the addition of the 30-kDa and 120-kDa fragments. The addition of the 45-kDa fragment resulted in a dose-dependent decrease in cell number. At the highest concentration of 45-kDa Fn-f examined, cell number fell below that of wells containing medium alone (Fig. 5). The proliferative effect was evaluated only at 24 h exposure, because at 48–72 h the autocrine production of growth factors by these cells makes interpretation of the effect of the Fn-f difficult. The 120-kDa fragment and 30-kDa fragment did not stimulate an increase in cell number at 24 h in HUVEC (data not shown).

Migration studies. The three fragments examined above were tested in modified Boyden chambers. The 30-kDa fragment showed a dose-dependent increase in HREC migration (Fig. 6*A*). Similar results were observed with the 120-kDa fragment (Fig. 6*B*). The 45-kDa fragment also induced migration in a dose-dependent fashion (Fig. 6*C*). The chemotactic response observed with the 120-kDa fragment and the 45-kDa fragment exceeded the response observed with equal molar concentrations of intact Fn, whereas the effect of Fn on HREC migration was not significantly different from that observed with the 30-kDa Fn-f (data not shown). Interestingly, the response observed with the 120-kDa and 45-kDa Fn-f was greater than the response observed with equal molar concentrations of bFGF (Fig. 6*B* and *C*). Also, the fragments induced significant amounts of migration at 4 h of exposure, whereas the bFGF response required 8 h of exposure for optimal response in this system (data not shown). The chemokinetic effect of the 30-kDa, 45-kDa, and 120-kDa fragments was evaluated by checkerboard analysis and was not significant.

DISCUSSION

Capillary morphogenesis involves the orchestrated effects of growth factors that modulate endothelial cell proliferation, migration, and tube formation. Mesenchymal precursors are recruited to encase the endothelial tube. The precursors differentiate into pericytes, and there is inhibition of endothelial proliferation, resulting in a mature capillary. Growth factors and ECM proteins can modulate each of these steps in capillary formation.

The ECM protein Fn is present in the walls of vessels, is concentrated at pericyte endothelial contacts (12), and is also present in the internal limiting membrane of the adult retina. Although not all studies have been able to detect Fn in the basement membrane of capillaries, the inability to do so may have been due to the type of fixation used (12), to the fact that postembedding immunogold techniques were performed (13), or to the fact that antigen retrieval techniques needed for many basement membrane proteins were not used. Others who did find Fn used a variety of more sensitive

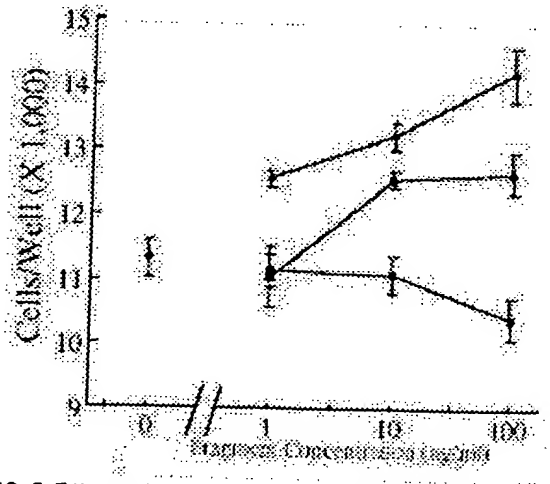

FIG. 5. Effect of 30-kDa tryptic (▲), 45-kDa tryptic (■), and 120-kDa chymotryptic (●) Fn-f on HREC proliferation as measured by change in cell number. Cells were plated in 24-well multiwell plates, then made quiescent by withdrawing serum for 18 h before adding Fn-f at the doses indicated. Serum-free medium was used as negative control (◆). After an additional 24 h of incubation, the cells were enzymatically dissociated and each well counted separately using a Coulter counter. Each point is the mean of four determinations. Error bars show standard error. Both the 30-kDa and 120-kDa Fn-f induce cell proliferation, whereas the 45-kDa fragment inhibits proliferation.

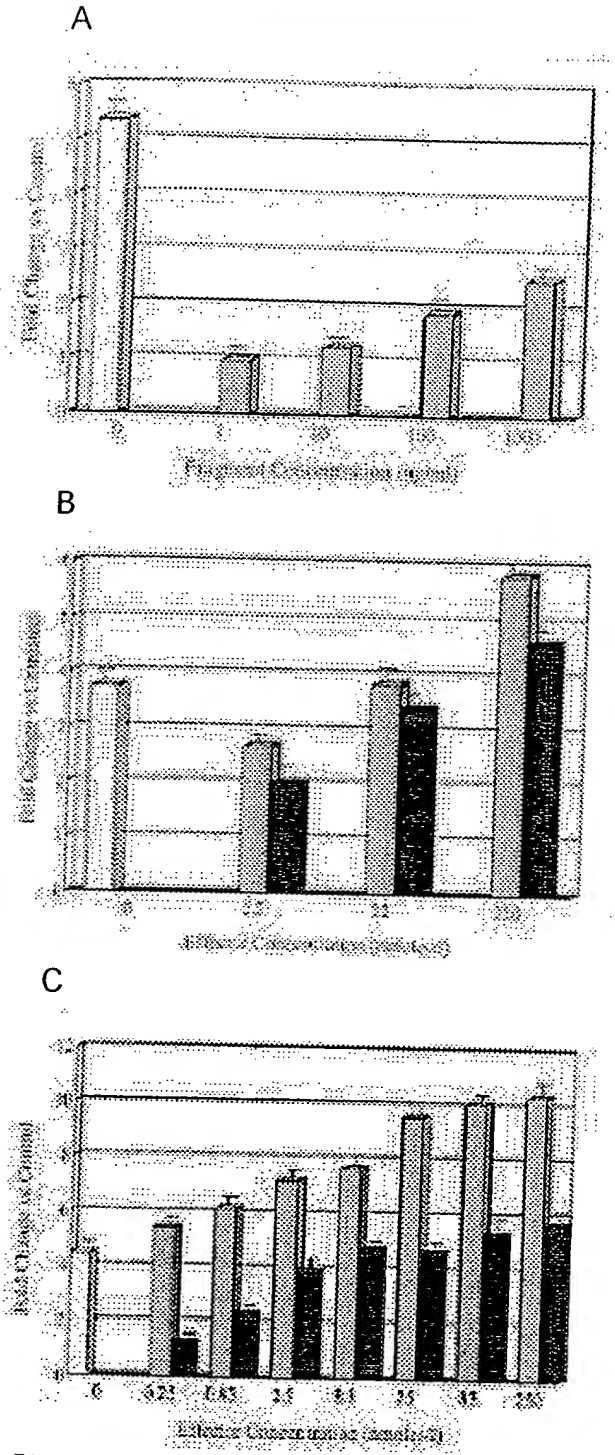

FIG. 6. Effect of Fn-f on migration of HREC, as measured using modified Boyden chambers. All three fragments induced dose-dependent migration of cultured microvascular endothelial cells. In all cases, medium containing 10% (wt/vol) fetal bovine serum was used as positive control. Each bar represents the mean of eight determinations, with error bars denoting standard error. All data were normalized to unstimulated cells (media alone) and expressed as fold change in the number of cells migrating. *A*: The 30-kDa fragment induces up to a 2.5-fold increase in migration at the highest dose tested, about half that induced by media + fetal bovine serum (□ on all three parts). The dose-dependent migration induced by either the 45-kDa (*B*, ▨) or the 120-kDa (*C*, ▨) fragments is actually greater (at maximally tested doses) than that induced by either media + fetal bovine serum or equal molar concentrations of bFGF (*B* and *C*, ■). In all cases, the data represented by each bar are significantly different ($P < 0.05$) from the previous dose.

techniques, including immunofluorescence and immunoperoxidase (14,15).

Immunofluorescence studies after trypsin digestion of the retina showed increased Fn immunoreactivity in large vessels and microvessels of patients with diabetes compared with control subjects. In situ hybridization studies of the trypsin-digested retinas from these diabetic patients showed Fn mRNA in the retina and retinal microvessels, a direct argument in support of local synthesis of Fn in the vessels of the human retina (16).

Using electron microscopy immunogold studies, we observed increases in Fn immunoreactivity in capillary basement membranes of rats with spontaneous diabetes compared with age-matched nondiabetic controls (17). In addition, the basement membrane zone of many new blood vessels is positive for an oncofetal Fn isoform containing the ED-B domain, a marker of angiogenesis (18,19).

Previous studies from our laboratory have shown that HRECs of diabetic origin expressed increased amounts of Fn compared with HRECs of nondiabetic origin. Exposure of HRECs of nondiabetic origin to high glucose increased the amount of Fn protein expressed (20). The diabetes-induced overexpression of Fn by endothelial cells is not readily reversible, and high glucose can mimic this effect in endothelial cells of nondiabetic origin. After six or seven cell replications, the glucose-induced elevation in Fn and type IV collagen is greater than in control cells (21). The differences we observed in HRECs of diabetic and nondiabetic origin give support to the finding that the events occurring during a finite period of metabolic derangement can leave long-lasting sequelae in the system (8). Processes that could propagate a "memory" of the diabetic state include hyperglycemia-induced irreversible modifications of long-lived ECM proteins. Although some effects of diabetes can be reversed by adequate insulinization, Fn overexpression and increased synthesis of glomerular basement membrane collagen do not decrease with treatment of diabetes. Increased Fn synthesis is also observed in fibroblasts explanted from diabetic mice and passaged in culture (22).

Proteolysis of Fn occurs near cells undergoing neoplastic transformation (23). Tumor cells elaborate proteases that can cleave Fn, including plasmin (24–26). Fn-f have been identified at sites of inflammation, injury, and destruction by metastatic tumor cells (27–31). Fn-f have been found to have activities not found in the intact molecule. Selected Fn-f have been shown to affect proliferation (27,28) and promote the adhesion (30,31), spreading, and migration of vascular endothelial cells (4–6,31,32).

Fn-f also induce expression of various proteases, including elastase (33), stromelysin (34), and metalloproteases (35,36). Studies by Imhoff et al. (37) demonstrated that a 190-kDa fragment of Fn produced by cathepsin D proteolysis in the presence of Ca^{2+} undergoes spontaneous autolysis, generating two enzymes, Fn-gelatinase and Fn-laminase, specific for the degradation of the ECM proteins, laminin, and Fn.

Recent studies have shown that Fn-f derived from residues 196–203 are potent stimulators of plasminogen activation catalyzed by tissue-type plasminogen activator (tPA) (38). Fn-f increased the efficacy of the plasminogen substrate. This region of Fn was within the fifth type-1 repeat in the NH_2-terminal domain of Fn. The primary physiological role of type-1 repeats is to bind fibrin, which

enhances the catalytic activity of tPA. Thus, interaction of plasminogen and tPA with ECM components may provide a fine regulatory mechanism for localized generation of plasmin proteolytic activity within the ECM.

Previous studies in our laboratory showed that HRECs express tPA in the quiescent state and urokinase when wounded in culture, and that plasmin activity was easily detectable in the conditioned medium of HRECs under basal conditions (8). The generation of plasmin can lead to activation of proforms of MMPs, and plasmin can degrade Fn, as can MMP-2. We have recently shown that HRECs produce MMP-2 (7). Fn-f could be generated in vivo at sites of angiogenesis by proteases secreted by endothelial cells (e.g., plasmin and MMPs) and/or mast cells (e.g., tryptase) (39).

The present study shows that the 120-kDa fragment increases DNA synthesis and cell number, but only in capillary endothelial cells, as this effect was not observed in HUVEC. The effect of the 30-kDa Fn-f is also specific to endothelial cells of the microvasculature, as the fragment did not affect HUVEC proliferation. We observed that selected fragments (45-kDa Fn-f) had opposite effects on HRECs and pericytes, a response that would be particularly beneficial if inhibition of endothelial cell proliferation was desired at the same time pericyte proliferation was required. For example, during the final stages of angiogenesis, the endothelial tube is already formed, but pericytes must still migrate and proliferate to encase this endothelial tube to complete the formation of the capillary. We have also observed that the Fn-f are more potent and act more quickly than bFGF on endothelial cell migration.

In summary, this study and previous work from our laboratory provide unique evidence for specific Fn-f regulating both cell movement and cell proliferation and activation of latent MMP-2 (7). Fn and Fn-f have potentially competing actions on steps relevant to angiogenesis. These studies support a complex regulatory role of Fn and its various domains, obtained by proteolytic degradation of Fn, in angiogenesis. The modulation by glucose of ECM components, such as Fn, may modify cellular behavior that may be specifically relevant to angiogenesis in diabetic retinopathy.

ACKNOWLEDGMENTS

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reference § 2

Gluconeogenesis, glucose ...

Gluconeogenesis, glucose production and fasting glycaemia

Original article:
Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans. A quantitative study. Gastaldelli A, Baldi S, Pettiti M et al. Diabetes 2000; 49: 1367-73.

Summary

Increased endogenous (hepatic and possibly renal) glucose production is thought to be a major factor regulating fasting glycaemia in Type 2 diabetes. Whether it relates to enhanced gluconeogenesis, elevated glycogenolysis, or both, is debatable. Little information is available on the effects of obesity per se on endogenous glucose production.

In the present study, fasting endogenous glucose fluxes and glycaemia were measured in 40 obese subjects (28 with Type 2 diabetes and 12 non-diabetic controls) and 15 non-obese subjects (nine with Type 2 diabetes and six non-diabetic controls). Endogenous glucose production was measured using 6,6-di-deuterated glucose, and gluconeogenesis by the 2H2O technique [1].

Several interesting observations were made. First, gluconeogenesis was increased in obese non-diabetic subjects. Second, gluconeogenesis was markedly increased in both obese and non-obese diabetic subjects. Third, gluconeogenesis and endogenous glucose production were both positively correlated with fasting glycaemia (Fig. 1). And, finally, hyperglucagonaemia was present in diabetic patients and was positively correlated with glucose fluxes.

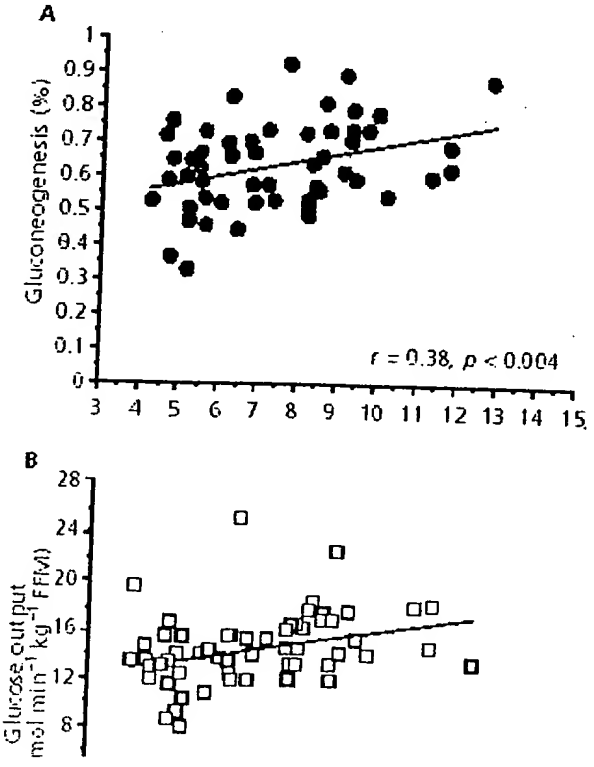

Fig. 1: Linear relationship between fasting plasma glucose and percent gluconeogenesis (A) and endogenous glucose output (B).

Comment

The fact that increased glucose production plays an important role in the pathogenesis of fasting hyperglycaemia in Type 2 diabetes is well documented. However, the mechanisms responsible for this increased glucose production are unknown. Several factors may be involved, including hepatic or renal resistance to the suppressive actions of both glucose and insulin on glucose production, increased secretion of

counterregulatory hormones, intrinsic dysregulation of hepatic/renal pathways of glucose production, or extrahepatic consequences of insulin resistance. Among the latter, increased lipolysis and proteolysis may stimulate gluconeogenesis by increasing gluconeogenic substrate availability and by enhancing plasma free fatty acid concentrations. High plasma free fatty acid concentration may in turn enhance hepatic gluconeogenesis and glucose output [2].

Obesity per se is also characterized by elevated rates of lipolysis and hence of glycerol and fatty acid release by adipose tissue. It may therefore be a major factor in enhanced glucose production under special circumstances. The present study carefully measured glucose production and gluconeogenesis in a large group of subjects including obese and non-obese individuals. The several pitfalls associated with measurements of glucose fluxes were skilfully avoided. The results obtained in obese subjects corroborate our earlier observations, i.e. that obesity per se stimulates gluconeogenesis without altering total glucose output [3, 4]. It has been recognized for several years that stimulation of gluconeogenesis can be attained by infusion of gluconeogenic precursors such as glycerol [5] or lactate [6]. Under such circumstances, an autoregulatory mechanism within the liver appears to prevent an increase in glucose production by simultaneously reducing net glycogenolysis [7]. This study clearly indicates that this autoregulatory mechanism is intact in obese non-diabetic patients.

In obese Type 2 diabetics, the picture, however, was quite different. Gluconeogenesis was further enhanced, possibly related to more severe extrahepatic and hepatic insulin resistance, and resulted in an increased glucose-6-phosphate flux in glucose-producing cells. Furthermore, hyperglucagonaemia was present, and is likely to play a major role in increasing glucose production, possibly by stimulating glucose-6-phosphatase. Last, but not least, both increased glucose production and whole body insulin resistance (documented by lower glucose clearance in diabetic patients) contributed to the development of hyperglycaemia.

What can we learn from these data? First, increased gluconeogenesis is not a sufficient factor per se to produce fasting hyperglycaemia. This suggests that strategies aimed at reducing gluconeogenesis may be ineffective in reducing glycaemia in Type 2 diabetes. This conclusion appears to be supported by the observation that acute ethanol administration did not lower glycaemia, although it suppressed gluconeogenesis in Type 2 patients [8]. Second, additional factors are present in Type 2 diabetes to stimulate glucose production. Hyperglucagonaemia may be one such factor, but other hormones or cytokines as well as neural factors may also be involved. Identification of such factors and of their mode of action may point to novel potential therapeutic strategies to reduce glucose production and fasting hyperglycaemia in Type 2 diabetes.

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Summary and Comment:
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED: 12/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Notification of Non-Compliant Appeal Brief (37 CFR 41.37)	Application No. 09/991,796	Applicant(s) JACKOWSKI ET AL.	
	Examiner Olga Chernyshev	Art Unit 1649	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

The Appeal Brief filed on *11/13/06* is defective for failure to comply with one or more provisions of 37 CFR 41.37.

To avoid dismissal of the appeal, applicant must file anamended brief or other appropriate correction (see MPEP 1205.03) within **ONE MONTH or THIRTY DAYS** from the mailing date of this Notification, whichever is longer. **EXTENSIONS OF THIS TIME PERIOD MAY BE GRANTED UNDER 37 CFR 1.136.**

1. ☒ The brief does not contain the items required under 37 CFR 41.37(c), or the items are not under the proper heading or in the proper order.

2. ☐ The brief does not contain a statement of the status of all claims, (e.g., rejected, allowed, withdrawn, objected to, canceled), or does not identify the appealed claims (37 CFR 41.37(c)(1)(iii)).

3. ☐ At least one amendment has been filed subsequent to the final rejection, and the brief does not contain a statement of the status of each such amendment (37 CFR 41.37(c)(1)(iv)).

4. ☐ (a) The brief does not contain a concise explanation of the subject matter defined in each of the independent claims involved in the appeal, referring to the specification by page and line number and to the drawings, if any, by reference characters; and/or (b) the brief fails to: (1) identify, for each independent claim involved in the appeal and for each dependent claim argued separately, every means plus function and step plus function under 35 U.S.C. 112, sixth paragraph, and/or (2) set forth the structure, material, or acts described in the specification as corresponding to each claimed function with reference to the specification by page and line number, and to the drawings, if any, by reference characters (37 CFR 41.37(c)(1)(v)).

5. ☐ The brief does not contain a concise statement of each ground of rejection presented for review (37 CFR 41.37(c)(1)(vi))

6. ☐ The brief does not present an argument under a separate heading for each ground of rejection on appeal (37 CFR 41.37(c)(1)(vii)).

7. ☐ The brief does not contain a correct copy of the appealed claims as an appendix thereto (37 CFR 41.37(c)(1)(viii)).

8. ☐ The brief does not contain copies of the evidence submitted under 37 CFR 1.130, 1.131, or 1.132 or of any other evidence entered by the examiner **and relied upon by appellant in the appeal**, along with a statement setting forth where in the record that evidence was entered by the examiner, as an appendix thereto (37 CFR 41.37(c)(1)(ix)).

9. ☐ The brief does not contain copies of the decisions rendered by a court or the Board in the proceeding identified in the Related Appeals and Interferences section of the brief as an appendix thereto (37 CFR 41.37(c)(1)(x)).

10. ☒ Other (including any explanation in support of the above items):

(1) Appeal Briefs are no longer filed under C.F.R 1.192 ,but C.F.R. 41.37
SECTION IX ,X: Brief does not contain the headings (ix) Evidence Appendix and (x) Related Proceedings Appendix. These sections must have a separate heading within the brief with a note of "None" if there are no Related Proceedings or Evidence present. Signature block and signature of attorney of record or authorizing offical is missing .

/ Everett R. Williams/
Everett R. Williams
Patent Appeals Specialist